Each homework consists of 3 problems, and you are expected to spend 30 min to 1 hour on each problem, but definitely less than 1 hour. If you find yourself spending more than 1 hour, you are probably overthinking about it. The optional problems may take significantly longer, so you can skip if you are short on time. But if you are interested in exploring further, the fun you get from working on the optional problems is definitely worth it!

1 Adaptation via reaction orders

We learned that although our engineered systems tend to use integral feedback as part of the controller to achieve perfect adaptation, biomolecular networks seem to use incoherent feedforward loops (IFFLs) more frequently. We explore the general distinction between the structures of these systems, and reconcile our observations with the control theory result that for a system to achieve perfect adaptation it is necessary and sufficient to have integral feedback. This is largely based on the work [1] and [2]. Using integral feedback to analyze biological adaptation was first done in [3] in the context of chemotaxis.

1.1 Adaptation, feedback versus feedforward, in linear control systems

We know that even for nonlinear dynamical systems, their dynamics around a fixed point can be analyzed and understood by a linear system obtained from linearization with respect to the fixed point. This is even more powerful for control systems, since the linear approximation would be all we need if our controller works as intended and controls the system to stay around the fixed point we linearized with respect to. In this case, the linear approximation always holds, so that although the underlying system is indeed nonlinear, the system dynamics never "see" it unless our controller fails. This is another reason why linear systems is often assumed by default in control theory and not considered a severe limitation. We will also see that this is the case for our understanding of biological systems. Linearization is great at revealing structures and can yield powerful insights. So our ability to analyze some biological phenomena via linear systems is a feature, not a limitation.

Coming back to our goal of understanding the general structure of feedback versus feedforward for adaptation, let us start with analyzing them for a linear (time invariant) control system with a single input and a single output (SISO).

$$\begin{aligned} \frac{d}{dt}x &= Ax + bw, \\ y &= cx + dw. \end{aligned} \tag{1}$$

Here $x \in \mathbf{R}^n$ is the vector of state variables, $w \in \mathbf{R}$ is the scalar disturbance input, and $y \in \mathbf{R}$ is the scalar output. So the shape of the dynamics matrices are $A \in \mathbf{R}^{n\times n}$, $b \in \mathbf{R}^{n\times 1}$, $c \in \mathbf{R}^{1\times n}$, and $d \in \mathbf{R}^{1\times 1}$.

We can also write this in block-matrix form which is more succinct.

$$\begin{bmatrix} \dot{x} \\ y \end{bmatrix} = \begin{bmatrix} A & b \\ c & d \end{bmatrix} \begin{bmatrix} x \\ w \end{bmatrix} = M \begin{bmatrix} x \\ w \end{bmatrix}, \tag{2}$$

where we define M to be the matrix with (A, b, c, d) as blocks.

1. Show that, at steady state,
$$y = (d - cA^{-1}b)w. \tag{3}$$

2. To achieve perfect adaptation, i.e. $y = 0$ for all constant w, we need
$$d - cA^{-1}b = 0. \tag{4}$$

If $d \neq 0$, draw a picture (control diagram) about how w maps to y via two branches. Reason that $d \neq 0$ corresponds to an incoherent feedforward (IFF) network motif where two branches cancel.

Therefore, for this to happen in an engineered control system, we need d to perfectly match $cA^{-1}b$. This requires physical parameters to satisfy exact equality, which is impractical.

Note that when $d = 0$, the perfect adaptation condition becomes $cA^{-1}b = 0$, and the structure of the network is hidden in A, so whether it is IFF or feedbcak is not clear.

3. (Optional.) Use the Schur complement formula for determinant to show that

$$\det \begin{bmatrix} A & b \\ c & d \end{bmatrix} = \det(A)(d - cA^{-1}b). \tag{5}$$

Since $\det A \neq 0$ for a stable system, we have $d - cA^{-1}b = 0$ is equivalent to $\det M = 0$. Checking whether a matrix's determinant is zero is fast computationally, so this can be done for very large scale systems.

4. In contrast to IFF, integral feedback (IFB) does not require fine tuning of parameters to achieve perfect adaptation. Consider $k = cA^{-1}$ and $z = kx$. Show that, under perfect adaptation, i.e. $d - cA^{-1}b = 0$, z satisfies

$$\dot{z} = y, \tag{6}$$

so z is an integral variable. Check that this holds even when $d = 0$, so the perfect adaptation condition becomes $cA^{-1}b$.

5. Consider the following example. This is adapted from Problem 2.3 in Homework 3.

$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \\ y \end{bmatrix} = \left[\begin{array}{cc|c} -k_{11} & -k_{12} & 1 \\ k_{21} & 0 & 0 \\ \hline 1 & 0 & 0 \end{array}\right] \begin{bmatrix} x_1 \\ x_2 \\ w \end{bmatrix}, \tag{7}$$

Here the parameters k_{11}, k_{12}, k_{21} are all positive. Check that this system achieves perfect adaptation, and write down on integral variable. Is this system using IFF or IFB?

1.2 Reaction order in the structure of biomolecular systems (Optional)

For a biomolecular system, we know variables x are concentrations, therefore positive. They also change via production and degradation reactions, which are processes with positive rates. The disturbance w is also reaction rate constants or species concentrations, which is positive. So we can write

$$\frac{d}{dt} x_i = f_i^+(x, w) - f_i^-(x, w), \tag{8}$$

for each variable x_i, where f_i^+ is the overall production flux of x, and f_i^- is the overall degradation flux of x.

To be consistent with the positivity, let us also define the output as

$$y = h(x, w) \tag{9}$$

and h is a positive function as well.

We linearize the system around a fixed point x^*, which satisfies $f_i^+(x^*) = f_i^-(x^*)$ for all i. This allows us to define a steady state parameter called the **lifetime** of species x_i, defined as

$$\tau_i^* = \frac{x_i^*}{f_i^\pm(x^*)}. \tag{10}$$

The interpretation is that τ_i^* is the steady state concentration of x_i^* divided by the production (or degradation) flux, so it gives the time it takes to re-produce all x_i molecules in the steady state pool by the steady state fluxes.

Since elementary reactions have monomial reaction rates, the production and degradation rates' exponents often have special structures. This is captured by log derivatives, also called **reaction orders**. We say the production (degradation) order of x_i with respect to x_j at point x^* is

$$H_{ij}^{A,\pm}(x^*) = \frac{\partial \log f_i^\pm}{\partial \log x_j}(x^*), \tag{11}$$

and the production-degradation order of x_i with respective to x_j at point x^* as $H_{ij}^A(x^*) = H_i^{A,+}j(x^*) - H_{ij}^{A,-}(x^*)$.

The log derivative ignores the multiplicative constants and extracts the exponents if $f_i^\pm$ is a monomial. For example, if $f_1^+ = k^+ x_1^{a_1} x_2^{a_2}$, then $H_{11}^{A,+} = a_1$ and $H_{12}^{A,-} = a_2$. If in addition $f_1^- = k^- x_2$, then $H_{11}^A = a_1$ and $H_{12}^A = a_2 - 1$.

Mimicking the definitions of (A, b, c, d), we can similarly define

$$\begin{aligned} H_i^{b,\pm} &= \frac{\partial \log f_i^\pm}{\partial \log w}, \\ H_i^b &= H_i^{b,+} - H_i^{b,-}, \\ H_j^c &= \frac{\partial \log h}{\partial \log x_j}, \\ H^d &= \frac{\partial \log h}{\partial \log w}. \end{aligned}$$

Since variables x, w, y are positive, we would like to linearize in a multiplicative fashion. So instead of the additive difference $\delta x_i = x_i - x_i^*$, we consider the multiplicative difference $\tilde{\delta} x_i = \frac{\delta x_i}{x_i^*} = \frac{x_i - x_i^*}{x_i^*}$. We do the same to define the local input and output variables $\tilde{\delta} w$ and $\tilde{\delta} y$ as multiplicative differences.

Show that the linearized system around fixed point x^* in terms of multiplicative differences satisfy

$$\begin{bmatrix} \frac{d}{dt}\tilde{\delta} x \\ \tilde{\delta} y \end{bmatrix} = \begin{bmatrix} \mathrm{diag}(\tau^*)^{-1} & 0 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} H^A & H^b \\ H^c & H^d \end{bmatrix} \begin{bmatrix} \tilde{\delta} x \\ \tilde{\delta} w \end{bmatrix}. \tag{12}$$

Here $\mathrm{diag}(\tau^*)$ is a diagonal matrix with the positive vector τ^* on the diagonal.

1.3 Reaction order condition for perfect adaptation

We mimic the analysis we did in the first subsection for adaptation in linear control systems, and apply it to the linearization of biomolecular system in Eqn 12 which has additional structure.

1. Consider the system in Eqn (12). Show that, at steady state,
$$\tilde{\delta} y = (H^d - H^c (H^A)^{-1} H^b) w. \tag{13}$$
(Hint: τ^* is a positive vector.)

   Try to map the results from the first subproblem to conditions on reaction order matrices (H^A, H^b, H^c, H^d).

2. Consider the following example of an IFF system achieving perfect adaptation.
$$\begin{aligned} \dot{x}_1 &= k_1 w - \alpha x_1 x_2, \\ \dot{x}_2 &= k_2 w - \beta x_2, \\ y &= x_1. \end{aligned} \tag{14}$$
This corresponds to the following biomolecular reaction network,
$$X_1 + W \xleftarrow{k_1} W \xrightarrow{k_2} W + X_2,$$
$$X_1 + X_2 \xrightarrow{\alpha} X_2 \xrightarrow{\beta} \emptyset.$$
(Optional.) Show that linearization of this system in terms of reaction orders yields the following:
$$\begin{bmatrix} H^A & H^b \\ H^c & H^d \end{bmatrix} = \left[\begin{array}{cc|c} -1 & -1 & 1 \\ 0 & -1 & 1 \\ \hline 1 & 0 & 0 \end{array}\right]. \tag{15}$$

3. Check that, assuming the system has a stable fixed point, the system achieves perfect adaptation just from conditions on the reaction orders, namely the determinant of the H^M matrix with (H^A, H^b, H^c, H^d) as blocks is zero.

   What's the integral variable?

   This provides an explanation for why biological system can routinely use IFF as a way to achieve perfect adaptation. Indeed, IFF requires parts of the system dynamics to perfectly match. But for most linear systems in engineered machines, the parameters that need to be fine-tuned to match perfectly are not structural, such as resistance and capacitance. These parameters are like concentrations, reaction rate constants, and lifetimes in biomolecular systems. However, due to the reaction order structure in the nonlinearity, biomolecular reaction networks can achieve perfect adaptation in a structural fashion, without fine-tuning of parameters.

2 Regimes of biocircuits reveal different modes of failure

In the previous problem, we saw that, from an example biocircuit, we could analyze the reactions to find out that the structure achieving perfect adaptation is contained in the reaction orders of the reactions. In this problem, we consider the relation in reverse. For a given reaction order structure achieving perfect adaptation, there could be several reaction networks having the same reaction order structure. Do these different implementations of the same reaction order structure make a difference? If so, in what sense? Combining the method of dominance regimes from our previous homework to analyze bioregulation, we could get an answer for this problem.

1. Consider the incoherent feedforward circuit below, which was studied in Problem 1.

$$\begin{aligned}\dot{x}_1 =& k_1 w - \alpha x_1 x_2,\\ \dot{x}_2 =& k_2 w - \beta x_2,\\ y =& x_1.\end{aligned} \tag{16}$$

This corresponds to the following biomolecular reaction network,

$$X_1 + W \xleftarrow{k_1} W \xrightarrow{k_2} W + X_2,$$
$$X_1 + X_2 \xrightarrow{\alpha} X_2 \xrightarrow{\beta} \emptyset.$$

Since X_2 here catalyzes the degradation of X_1, from the binding-catalysis perspective of bioregulation, we know the underlying regulatory mechanism must be that X_1 and X_2 binds to form a complex before X_1 is degraded. For a simple mechanism of this form, let us consider the following.

$$X_{1,f} + X_{2,f} \overset{K}{\rightleftharpoons} C_{12} \xrightarrow{k_{\text{cat}}} X_{1,f}. \tag{17}$$

Here K is the dissociation constant, $X_{i,f}$ is the X_i molecule free in solution, where $i = 1, 2$, and C_{12} is the complex formed by X_1 binding with X_2. The previous reactions involving X_1 and X_2, without the f subscript, means they do not distinguish the free versus bound forms of X_1 and X_2 molecules, and they all participate.

So, to be consistent with this notation, the total concentration of X_1 in the context of this binding reaction is $X_1 = X_{1,f} + C_{12}$, and similarly $X_2 = X_{2,f} + C_{12}$ is the total concentration of X_2.

Notice that the binding reaction between $X_{1,f}$ and $X_{2,f}$ is just like the enzyme-substrate binding reaction $E + S \rightleftharpoons C$. What is the regime that achieves the perfect adaptation reaction order, $C_{12} \propto X_1 X_2$? What is the (asymptotic) validity condition in terms of X_1, X_2, K?

Combine this with the steady state relation $x_2^* \propto w$. Does this mean the system will fail, i.e. go out of the validity condition of the adaptive regime, for disturbance w that is too large or too small?

2. We can consider another incoherent feedforward circuit with X_2 repressing the production of X_1.

$$\begin{aligned}\dot{x}_1 =& k_1 \frac{w}{x_2} - \alpha x_1,\\ \dot{x}_2 =& k_2 w - \beta x_2,\\ y =& x_1.\end{aligned} \tag{18}$$

Show that this system has the same reaction order matrices (H^A, H^b, H^c, H^d) as the degradation one considered above, therefore achieves perfect adaptation.

3. For the repression IFF above, we can consider the following biomolecular reaction network achieving it:

$$W \xrightarrow{k_2} W + X_2,$$
$$X_1 \xrightarrow{\alpha} \emptyset \xleftarrow{\beta} X_2,$$
$$W_f + X_2 \overset{K}{\rightleftharpoons} C_{WX_2}, \;\; W_f \xrightarrow{k_1} W_f + X_1.$$

Here X_1 and X_2 degrades by themselves, and W catalyzes the production of X_2. W also catalyzes the production of X_1, but this is repressed by X_2, so only the free form of W that is not bound by X_2, denoted W_f, is active in producing X_1.

Consider the binding reaction between W_f and X_2, and the active species W_f as output. What is the regime that achieves the desired reaction order? What is the validity condition for this regime?

Together with the steady state relation, $x_2 \propto w$, what does this imply about the disturbances w that this system will fail to adapt? w that is too large or too small?

Reference: The degradation IFF design is often called the sniffer model, and is discussed as a classic example of adaptation in systems biology. For example, see [4] for a brief review. The repression IFF was experimentally implemented to achieve adaptation to variations in the number of plasmids in [5]. Very recently, [6] attempted to use the same principle to design micro RNA circuits that can compensate for gene dosage changes in mammalian cells.

3 Integral windup in integral feedback

Although both incoherent feedforward (IFF) and integral feedback (IFB) can achieve perfect adaptation in biological systems, IFB has a unique advantage that its adaptation property does not depend on the details of the plant! The integral control variable is solely in the controller, therefore by its existence, guarantees that if the system reaches steady state, it achieves perfect adaptation. In other words, it could be argued that IFB achieves an extremely robust form of perfect adaptation that is independent of all the details of the plant!

This may make it seem like IFB is always much more desirable. This is especially the case for engineered machines, and for engineered biological systems in synthetic biology. Because from a human design perspective, modularity facilitates iterative improvements. IFB makes it possible to have an “adaptation module” that could be attached to any plant and achieve adaptation.

However, it is already understood in control theory that the reality is more complicated. On one hand, perfect adaptation requires both an integral variable and that the system achieves steady state. Although IFB guarantees the former independent of plant details, the latter, which involves stability, is always complicated and depends on the plant. In fact, by attaching IFB to a plant, it usually tends to make the plant less stable, or harder to stabilize.

Of course, in biology, we tend to downplay the importance of stability since cells always seem stable, or we simply would not see unstable cells since they would not survive and grow. So we may care more about limitations of IFB other than stability.

On the other hand, IFB, because of its feedback and error-accumulation nature, may result in a phenomenon called integral windup. The idea is that, linear systems are always only valid in a finite region, and when a variable becomes too large, the linear assumption would not hold, and nonlinearity such as saturation would start to appear. The error accumulation could result in the integral variable quickly become very large when the disturbance is strong, so that the feedback actuation may be saturated and no longer responsive.

We explore the consequence of integral windup in this problem.

1. Consider the following perfect adaptation circuit with integral feedback.

$$\begin{aligned} \dot{x}_1 &= \alpha x_2 - \gamma x_1, \\ \dot{x}_2 &= w - x_1, \\ y &= w - x_1. \end{aligned} \tag{19}$$

Here x_1 is positive, but x_2 is real. w is disturbance input, positive, while $y = w - x_1$ is output.

Is the system adaptive? What is the integral variable?

Simulate the system's dynamics, with w taking a square wave, i.e. stay at a low value for a duration, and then stay at a high value for a duration, and repeat. Observe that the system adapts perfectly if allowed to reach towards steady state.

2. Now let us introduce saturation. Saturation often happens because the system needs to be physically implemented. Our above equation for dynamics is a linear system, and x_2 is an integral variable of x_1. But to achieve this with biomolecular reactions, x_2's dynamics cannot be exactly implemented as the concentration of a species.

   Let us consider the following implementation so that x_2 is a virtual variable, $x_2 = x_2^+ - x_2^-$.

$$\begin{aligned} \dot{x}_1 &= \alpha x_2^+ - \gamma x_1, \\ \dot{x}_2^+ &= w - kC, \\ \dot{x}_2^- &= x_2 - kC, \end{aligned} \tag{20}$$

   where C is the concentration of the complex from the following binding reaction,

$$X_{2,f}^+ + X_{2,f}^- \overset{K}{\rightleftharpoons} C \to \emptyset, \tag{21}$$

   where $X_{2,f}^{\pm}$ are the free form of $X_2^{\pm}$ molecules in the solution.

   Observe that, if the degradation of $X_2^{\pm}$ by C is fast, then only one of X_2^+ and X_2^- is positive at any given time. Show that, under this assumption and defining $x_2 = x_2^+ - x_2^-$, we have the following dynamics,

$$\begin{aligned} \dot{x}_1 &= \alpha \max x_2, 0 - \gamma x_1, \\ \dot{x}_2 &= w - x_2. \end{aligned} \tag{22}$$

   The production rate of x_1 now saturates if x_2 becomes negative. This is saturation in the actuation of the integral feedback by x_2.

3. Simulate the above system with actuator saturation. Play around with the magnitude and durations of the square wave input w. Observe that, for certain parameters, the controller may "skip" a period of the disturbance and does not track like before. Explain why this happens based on the dynamics of x_2.

   This "period skipping" shows that when integral windup happens, the huge magnitude of the integral variable may render the system irresponsive for a while, causing detrimental influences on system performance.

4. (Optional.) Simulate one of the incoherent feedforward systems in the previous problems and observe that they do not have integral windup or period skipping.

   This "period skipping" behavior is suggested as a method to distinguish feedback versus feedforward systems in [7].

5. (Optional.) If you try to fix the integral windup problem, what would you do?

   Here is one suggestion. Instead of implementing x_2 as the difference between two virtual variables, what if we let x_1 catalyze the degradation of x_2 and operates in a regime so that x_2 saturates and the degradation rate is independent of x_2? This means the following reaction:

   $$X_2 + X_1 \rightleftharpoons C_{12} \to X_1.$$

   Under what regime does this reaction implement the desired integral feedback system? What is the validity condition? When does this reaction violate the validity condition and saturates?

   This situation is a sensor saturation, since when x_2 is too small, the degradation rate of x_2 becomes sensitive to x_2 again, so x_2 no longer serves as an integral variable.

   Does the integral windup behavior still happen in this case?

References

[1] F. Xiao and J. C. Doyle, "Robust perfect adaptation in biomolecular reaction networks," in *2018 IEEE Conference on Decision and Control (CDC)*, 2018, pp. 4345–4352.

[2] F. Xiao, M. Khammash, and J. C. Doyle, "Stability and control of biomolecular circuits through structure," in *2021 American Control Conference (ACC)*, 2021, pp. 476–483.

[3] T.-M. Yi, Y. Huang, M. I. Simon, and J. Doyle, "Robust perfect adaptation in bacterial chemotaxis through integral feedback control," *Proceedings of the National Academy of Sciences*, vol. 97, no. 9, pp. 4649–4653, 2000. [Online]. Available: https://www.pnas.org/doi/abs/10.1073/pnas.97.9.4649

[4] J. E. Ferrell Jr., "Perfect and near-perfect adaptation in cell signaling," *Cell Systems*, vol. 2, no. 2, pp. 62–67, Feb 2016. [Online]. Available: https://doi.org/10.1016/j.cels.2016.02.006

[5] T. H. Segall-Shapiro, E. D. Sontag, and C. A. Voigt, "Engineered promoters enable constant gene expression at any copy number in bacteria," *Nat Biotechnol*, vol. 36, no. 4, pp. 352–358, Mar. 2018.

[6] R. Du, M. J. Flynn, K. Mahe, M. Honsa, B. Gu, D. Li, S. E. McGeary, V. Gradinaru, R. Jungmann, and M. B. Elowitz, "mirna modules for precise, tunable control of gene expression," *bioRxiv*, 2025. [Online]. Available: https://www.biorxiv.org/content/early/2025/07/16/2024.03.12.583048

[7] S. J. Rahi, J. Larsch, K. Pecani, A. Y. Katsov, N. Mansouri, K. Tsaneva-Atanasova, E. D. Sontag, and F. R. Cross, "Oscillatory stimuli differentiate adapting circuit topologies," *Nature Methods*, vol. 14, no. 10, pp. 1010–1016, Oct 2017. [Online]. Available: https://doi.org/10.1038/nmeth.4408